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(54) **METHODS AND COMPOSITIONS FOR RNA-DIRECTED TARGET DNA MODIFICATION AND FOR RNA-DIRECTED MODULATION OF TRANSCRIPTION**

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This patent is subject to a terminal disclaimer.

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(58) **Field of Classification Search**

None

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,766,900 A 6/1998 Shillito et al.
5,767,367 A 6/1998 Dudits et al.
(Continued)

FOREIGN PATENT DOCUMENTS

CN 103224947 A 7/2013
CN 103233028 A 8/2013
(Continued)

OTHER PUBLICATIONS

Wu et al. (2009) Effect of Genome Size on AAV Vector Packaging. Molecular Therapy, 18(1):80-86.*
(Continued)

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(57) **ABSTRACT**

The present disclosure provides a DNA-targeting RNA that comprises a targeting sequence and, together with a modifying polypeptide, provides for site-specific modification of a target DNA and/or a polypeptide associated with the target DNA. The present disclosure further provides site-specific modifying polypeptides. The present disclosure further provides methods of site-specific modification of a target DNA and/or a polypeptide associated with the target DNA The present disclosure provides methods of modulating transcription of a target nucleic acid in a target cell, generally involving contacting the target nucleic acid with an enzymatically inactive Cas9 polypeptide and a DNA-targeting RNA. Kits and compositions for carrying out the methods are also provided. The present disclosure provides genetically modified cells that produce Cas9; and Cas9 transgenic non-human multicellular organisms.

13 Claims, 128 Drawing Sheets

Specification includes a Sequence Listing.